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The Aerial Parts of Yellow Horn Poppy (*Glaucium flavum* Cr.) growing in Egypt:Isoquinoline Alkaloids and Biological Activities

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Abstract

Glaucium flavum Cr. (Family: Papaveraceae), known as the yellow horn poppy, is a medicinal herb found in the Mediterranean region, including Egypt and southern Europe. The plant is well known for its pharmacologically active aporphine-type isoquinoline alkaloids. The plant also exhibits various medicinal properties including antitussive, antioxidant, hypoglycemic, and bronchodilator activities. *G. flavum* is now considered rare and endangered in the Egyptian flora due to urban sprawl and climate changes. The ethanol extract of the aerial part of the plant was subjected to acid–base extraction; the acidic methylene chloride fraction was further chromatographed to yield four isoquinoline alkaloids; glaucine, pontevedrine, oxoglaucine, and catalane. The ethanol extract of aerial parts of was investigated for its analgesic, anti-inflammatory, cytotoxic, antibacterial and antifungal activities. The extract reduced writhing in the acetic acid-injected rats by 84.62% compared to untreated animals (control), indicating good analgesic activity. The extract showed biological activity equivalent to diclofenac sodium as an anti-inflammatory agent using the rat hind paw edema method. The extract showed good cytotoxic activity on HepG2 and HCT cells with an IC_{50} of 28.3 µg and 33.2 µg, respectively. The antibacterial and antifungal activities of the aerial parts ethanolic extract were significant, especially among Gram-negative bacteria such as *E. coli*.

Keywords: Antimicrobial; anti-inflammatory; catalane; cytotoxic; glaucine; pontevedrine.

INTRODUCTION

The Poppy family (Papaveraceae) comprises about 23 genera distributed all over the world [1]; of these, four genera – *Papaver*, *Argemone*, *Roemeria*, and *Glaucium* – are reported in Egypt. The genus *Glaucium* features approximately 23 species that are primarily distributed in the Mediterranean region, Europe, southwestern and central Asia, and North America [2].

Glaucium flavum Cr. (Figure 1) is a perennial herb measuring up to 90 cm in height. The plant has different synonyms, such as the yellow horned poppy [3], yellow horn poppy, *G. luteum* L., and *Chelidonium glaucium* L. [4]. The name "horned-poppy" is derived from its very long, swollen, and pointed capsules, bearing horn-like protrusions [5]. The stems of *G. flavum* Cr. bear yellow poppy-like flowers from June to August; these flowers then give rise to elongated siliquiform capsules with seeds ripening from August to September. The yellow horned poppy is distributed in the Mediterranean regions, and it is also found along both the Atlantic coast of Europe and the Black Sea coast [3].

The Egyptian medicinal flora consists of about 342 species; it is distributed in 13 places and it can be divided into four groups: very common (91 species), common (86 species), rare (95 species), and very rare (70 species) [6, 7]. *G. flavum* began to recede in many parts of Europe [8], and it is now classified as a rare species of Egyptian flora (Batanouny, 2005); this may be the result of metropolitan extension and weather variations in the plant's natural habitat in the last decade, particularly along the northern coast. Egyptian authorities and several civil community organizations have made efforts to save these endangered and rare species. Examples of these initiatives include the establishment of the Desert Research Institute in Cairo (1990) and the development of the non-governmental organization medicinal plants conservation project, as supported by the National Conservation Sector in 2003, in the city of Saint Catherine. In addition, the issuance of various laws and regulations for the establishment and maintenance of nature reserves also took place; however, all of these efforts require an updated list of different endangered plants in the Egyptian flora.

G. flavum Cr. has previously been studied for its medicinal and pharmacological activities, including its antitussive, antioxidant [9, 10], hypoglycemic [11], and hypotensive effects [12]. This plant is characterized by its rich secondary metabolites and it has been reported to possess isoquinoline alkaloids such as the aporphine, oxoaporphine, benzylisoquinoline, protoberberine, tetrahydoprotoberberine, protopine, morphinan, and benzophenanthridine alkaloids [13].

This study describes the isolation of four isoquinoline alkaloids from the aerial parts of *G. flavum*, which are growing widely in Egypt. The biological activities of the plant extract, including its anti-inflammatory, analgesic, cytotoxic, and antimicrobial activities, have been explored.

Figure 1: The entire plant of *G. flavum*collected in November 2010 from Sidi Barrani, north coast, Egypt.

MATERIALS AND METHODS

Plant materials

The plant materials used in this work, *G. flavum* Cr. (Family: Papaveraceae), were collected on November 2010 from Sidi Barrani, at the north coast of Egypt. The plant was identified and verified by Prof. Dr Samih I. Eldahmy, Professor of Pharmacognosy, Faculty of Pharmacy, Zagazig University. Voucher specimens were deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt, under number P145.

Extraction and isolation

The air-dried powdered aerial parts of *G. flavum* Cr (1.1 kg) were repeatedly extracted with ethyl alcohol 70% (5×3 L) at room temperature. The ethanol extract residue of the aerial parts (182 g), which was obtained following evaporation of the solvent under reduced pressure, was fractionated through an acid–base extraction,. The residue (100 g) was suspended in a 5% aqueous solution of HCl (1 L) and extracted with methylene chloride (0.5 L × 6 extractions) to yield the acidic methylene chloride fraction, 29.30 g.

The acidic methylene chloride fraction was dissolved in chloroform–methanol (2:1), and crystallization was enabled to yield compound **1** (1.17 g).

The remaining acidic methylene chloride residue (25 g) was chromatographed on a silica gel column (90 × 5 cm; 600 g). Elution was attained using petroleum ether, to which methylene chloride and, subsequently, methanol were added in gradient mode.

The fraction eluted by 20% methanol in methylene chloride (15 g) was chromatographed on a silica gel column (90 × 3 cm; 400 g). Elution was achieved using methylene chloride, to which methanol was added in gradient mode. The fraction eluted using 5% methanol in methylene chloride (2 g) was chromatographed on an alumina column (150 g). The elution process started with methylene chloride, and the polarity was then gradually increased using methanol. Fractions eluted by 100% methylene chloride were allowed to crystallize to yield compound **2**. Compounds **3** and **4** were crystallized from 2.5% and 5% methanol in methylene chloride fractions, respectively. All nuclear magnetic resonance (NMR) experiments were carried out using a Bruker 600 MHz spectrometer, while mass spectra were carried out on a Shimadzu QP5050A mass spectrometer at 70 eV. All compounds were tested for purity using TLC (silica gel 60G F_{254} glass plates; Merck KGaA, Darmstadt, Germany);development was performed using chloroform: methanol: NH_4OH (39%) (9.5: 0.5: 2 drops);and visualization was accomplished using Dragendorff's reagent (for alkaloidal impurities) and 10% methanolic sulfuric acid (for non-alkaloidal impurities).

Compound 1 (Glaucine): 1.17 g, buff rosette crystals (chloroform/methanol), R_f = 0.74, positive to Mayer's and Dragendorff's reagents, EI-MS. m/z (rel.int, %): 355 (100, M^+); 341 (43.9); 339 (5); 324 (21.4); 298 (17.9); 282 (29.2); 265 (8.77); 251 (7.6); 238 (5.7); 223 (8); 210 (9.6); 195 (9.3); 180 (14.5); 165 (18.7); 152 (33.6); 139 (29.5); 127 (8.3); 111 (14); 97 (15.5); 83 (12.7); 71 (19.8); and 54 (68.8). ^{1}H-NMR and ^{13}C-NMR (DMSO-d_6, 600 MHz and 150 MHz): Table 1.

Compound 2 (Pontevedrine): 200 mg, red needles (chloroform–methanol), freely soluble in chloroform, R_f = 0.43, positive to Mayer's and Dragendorff's reagents. EI-MS. m/z (rel.int, %): 382 (100, M^++H); 367 (9); 354 (23.6); 339 (51.4); 324 (7.7); 308 (29); 295 (78); 280 (99.6); 265 (40); 251 (55.9); 236 (21.5); 223 (38.5); 209 (20.3); 194 (18); 180 (31.8); 164 (43); 152 (61.9); 138 (43); 125 (31.6); 111 (13.5); 89 (6.2); 75 (19.6); 62 (8.3); and 50 (5.8). ^{1}H-NMR and ^{13}C-NMR (DMSO-d_6, 600 MHz and 150 MHz): Table 1.

Compound 3 (Oxoglaucine or O-methylatheroline): 30 mg, orange crystals (chloroform-methanol). It is freely soluble in methanol, R_f = 0.16, with a positive response to Mayer's and Dragendorff's reagents. ^{1}H-NMR and ^{13}C-NMR (DMSO-d_6, 600 MHz and 150 MHz): Table 1.

Compound 4 (Catalane): 5 mg, white crystals (chloroform–methanol). It is freely soluble in chloroform, R_f = 0.22, and has a positive response to Mayer's and Dragendorff's reagents. EI-MS. m/z (rel.int, %): 370 (57.5, M^+-H); 355 (29); 341 (14); 324 (5.9); 311 (40); 281 (10.6); 265 (50); 235 (14); 224 (40.5); 210 (33.6); 194 (41.4); 178 (48.9); 164 (85.4); 151 (100); 139 (48.5); 126 (18.2); 90 (6.3); 75 (11.3); 70 (28.3); and 50 (9.1). ^{1}H-NMR and ^{13}C-NMR (DMSO-d_6, 600 MHz and 150 MHz): Table 1.

Pharmacological activities

Animals

Adult male albino rats weighing 200–220 g were used in the analgesic and anti-inflammatory studies. The animals were obtained from the animal house of the National Research Center, Dokky, Cairo, Egypt, and were treated according to the protocols set forth by the ethical committee of animal handling at Zagazig University (ECAHZU). All animals were held under standard laboratory conditions in the animal house of the Faculty of Pharmacy, Zagazig University under 12-hour light/12-hour dark cycles at 27°C. The animals were provided with excess food and water *ad libitum*.

Extract preparation

The previously prepared ethanol extract was used for analgesic and anti-inflammatory activity testing. The extract was suspended in 7% gum acacia and was orally administered to animals at a dose of 40 mg/kg. This dose was chosen after a routine pilot experiment, in which several doses of the extract (5, 10, 20, 40, 80, 200 and 500 mg/kg) was tried (in analgesic and anti-inflammatory experiments). The 40 mg/kg dose was chosen as the lowest dose to have an effect. Each dose was repeated three times to ensure reproducibility of the results.

Analgesic activity

The analgesic activity of the ethanol extract was determined using the acetic acid-induced writhing technique [14]. A sensitivity test for acetic acid was carried out 1 day before the experiment, during which each rat was injected intraperitoneally with 0.1 mL/10 g body weight of 0.6% acetic acid. The rats were observed for 15 minutes; the response in these animals manifested as contraction of the abdominal muscles and stretching of the hind limbs. The rats that exhibited writhing behavior were considered to have a positive response. After 24 hours of the sensitivity test, acetic acid-sensitive rats were divided into two groups (n=6). The first group was orally administered gum acacia mucilage (7%) and served as the control. The second group received the extract, suspended in 7% gum acacia, at a dose of 40 mg/kg orally. After 1 hour, acetic acid was injected and the number of writhes during the following 25-minute period in 5-minute intervals was counted.

Anti-inflammatory activity

The anti-inflammatory activity extract was studied using the hind paw edema method [15]; diclofenac sodium was used as the reference standard. Rats were divided into three groups (n=5): the first group was given gum acacia mucilage (7%) and served as a control. The second group received diclofenac sodium at a dose of 4 mg/kg, while the third group received the extract (40 mg/kg) suspended in 7% gum acacia. All treatments were orally administered 1.5 hours before the induction of inflammation. Inflammation was induced by the subcutaneous injection of 0.1 mL of carrageenan (1% suspension in saline) into the sub-plantar surface of the right hind paw of all animals. The left hind leg was injected with 0.1 mL of normal saline and served as a control. Hind paw size was measured just before the injection of carrageenan, as well as after 1, 2, 3, 4, 6, and 24 h. Hind paw thickness was measured for each rat at each time interval, and the mean induced edema thickness was calculated. The total area under the curve (AUC), representing edema thickness at various time intervals (%.hours) was calculated using the trapezoidal method [16]. The data were expressed as the mean ± standard error of mean (SEM) for five animals.

Cytotoxic activity

Human breast carcinoma cells (MCF-7), hepatocellular carcinoma cells (HepG2), colon carcinoma cells (HCT), cervical carcinoma cells (HeLa), and larynx epidermoid carcinoma cells (HEp-2) were used to evaluate the cytotoxic effects of the tested plant extract. The cells were routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM), which was supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid) buffer, and 50 µg/mL of gentamycin. The cells were maintained at 37°C in humidified air containing 5% CO_2 and they were sub-cultured two times a week. The extract was dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted to the required concentration. All experiments were repeated three times, unless otherwise indicated.

The cell viability assay technique was used to evaluate the cytotoxicity of the plant extract according to [17]. The cells were seeded in 96-well microliter plates at a cell concentration of 1×10^4 cells per well in 100 µL of growth medium containing serial dilutions of the tested plant extract (50, 25, 12.5, 6.25, 3.125, and 1.56 µg). The microliter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 hours. Three wells were used for each concentration of the test sample. The control cells were incubated without the test sample and with or without DMSO. Following incubation, the cells were fixed and stained with crystal violet solution (1%). The stain was removed and the plates were rinsed using tap water until all of the excess stain was removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and the absorbance was measured at 490 nm. The effect on cell growth was calculated as the difference in absorbance percentage in the presence and absence of the tested extracts;this was illustrated in a dose–response curve. The concentration at which cell growth was inhibited to 50% of the control (IC_{50}) was obtained from this dose–response curve [18].

Antimicrobial activity

The antibacterial and antifungal activities of the ethanol extract was determined using the well diffusion method [19]. Gram-positive bacteria (*Staphylococcus aureus* [RCMB 010028] and *Bacillus subtilis* [RCMB 010067]), Gram-negative bacteria (*Pseudomonas aeruginosa* [RCMB 010043], *Escherichia coli* [RCMB 010052], *Salmonella typhimurium* [RCMB 010072], and *Klebsiella pneumoniae* [RCMB 000111]), and fungi (*Aspergillus fumigatus* [RCMB 02568] and *Geotricum candidum* (RCMB 05097)] were the standard strains used in this study, and they were obtained from the Department of Microbiology, Faculty of Pharmacy, Zagazig University, Egypt. Ampicillin and gentamicin were used as a positive control against Gram-positive and Gram-negative bacteria, respectively, while amphotericin B was used as a positive control for fungi. The extract was dissolved in DMSO at a concentration of 1 mg/mL. The tested organisms were subcultured on nutrient agar medium for bacteria and Saboroud dextrose agar for fungi. Bacterial cultures were incubated at 37°C for 24 hours, while the fungal cultures were incubated at 37°C for 2–7 days. Antibacterial and antifungal activities were determined by measuring the inhibition zone diameter formed around the well (mm). The results were expressed in terms of the mean zone of inhibition in mm ± standard error of the mean (SE).

Statistical analysis

Quantitative values are expressed as the mean ± SE (unless otherwise specified), and significant differences were determined using Student's (unpaired) *t*-test. The analyses were performed using the SPSS statistical package (SPSS for Windows, version 11.5; SPSS Inc., Chicago, IL, USA). $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

The acidic methylene chloride fraction of the ethanol extract of the aerial parts of *G. flavum* Cr. was subjected to various isolation and structure elucidation techniques, as described in the materials and methods section. This afforded the isolation of four isoquinoline alkaloids (Figure 2).

Compound **1** showed positive chemical reactions with Mayer's and Dragendorff's reagents, suggesting it was an alkaloid[20]. The EI-MS exhibited a molecular ion peak at m/z 355 (100%), fitting nicely with molecular formula $C_{21}H_{25}NO_4$. The mass fragmentation pattern that was observed for compound **1** was in accordance with that previously reported for glaucine [21, 22]. The ^{1}H-NMR spectrum (Table 1) showed four methoxy groups occurring as singlets at δ3.83, 3.80, 3.77, and 3.62 ppm. A signal at δ 3.07 ppm (3H, s) was assigned to three protons of the $-NCH_3$ group. Aromatic protons appeared as singlets at δ 6.88, 6.99, and 7.87 ppm. Two doublets of doublets at δ 2.98 and 3.40 ppm were assigned to protons on carbons 4 and 5. A triplet at δ 2.70 ppm was assigned to proton 6a. These ^{1}H-NMR results are in agreement with the data published elsewhere for glaucine[22, 23]. The ^{13}C-NMR spectrum (Table 1) indicated the presence of 21 carbons, 12 of which were in the aromatic region and four were typical methoxy carbons. The aforementioned results, when compared with those in the available literature [21-23], suggested that compound **1** was glaucine. Glaucine was previously reported to be the main isoquinoline alkaloid present in *G. flavum*. It was suggested to possess antitussive[23-25], antioxidant [9, 10], antihypertensive[5, 26], antihyperglycemic [27], bronchodilator [28, 29], and antithrombotic activities. The antitussive effect of glaucine has many advantages over the currently available antitussive preparations, especially codeine, as it features reduced depressing activities on respiration, no inhibiting effect on intestinal motility, and no habit-forming or addictive properties[30].

The EI-MS spectrum of compound **2** showed a molecular ion peak at m/z 382 (100%), corresponding to molecular formula $C_{21}H_{19}NO_6$. The mass spectrum also contained fragments with m/z of 367 (9%) and 354 (23.6%), which corresponded to the loss of the methyl and carbonyl groups, respectively. The ^{1}H-NMR spectrum of compound **2**(Table 1) showed an absence of four protons, which presented at positions 4 and 5 in compound **1** (glaucine). The aromatic protons appeared as singlets at δ 7.61, 8.14, and 8.99 ppm. Four proton singlets at δ 3.94, 3.97, 4.07, and 4.08 ppm were assigned to the four methoxy groups. Olefinic proton appeared asa singlet at δ 7.86 ppm, representing the proton on carbon 7. The ^{13}C-NMR spectrum of compound **2**(Table 1) suggested the presence of a 21 carbon skeleton. Two carbonyl signals were present (δ 175.23 and 155.62 ppm) and assigned to carbons 4 and 5, respectively. Carbon 5 was slightly shielded due to its attachment to a shielding atom, nitrogen. When comparing the previously mentioned data with the available literature, Ribas et al. (1971) concluded that compound **2** was pontevedrine. This compound was isolated earlier from *G. flavum*[13, 31].

Compound **3** yielded a white precipitate and an orange–red color with Mayer's and Dragendorff's reagents, respectively, indicating the presence of an alkaloid. The ^{1}H-NMR spectrum of compound **3** (Table 1) showed the absence of two protons, which presented at position 7, indicating the presence of other groups at this position. The chemical shift of the proton at position 8 proved that the presence of an electron-withdrawing group was carbonyl at position 7. With respect to the aromatic protons, two proton doublets were noted at δ 8.08 and 8.84 ppm at positions 4 and 5, respectively,with a coupling constant of 4.8 Hz for each proton. Other aromatic protons appeared as singlets at δ 7.67, 7.82, and 8.75 ppm, while other singlets were identified at δ 3.94, 3.99, 4.02, and 4.06 ppm, each one integrated for three protons of the methoxy groups. The ^{13}C-NMR spectrum of compound **3** (Table 1) indicated the presence of 20 carbons. When comparing the ^{13}C-NMR spectrums of compounds **1** and **3**, it was suggested that the absence of the methyl group attached to the nitrogen atom from compound **3**. Comparing the aforementioned data with the available literature [32], it was suggested that compound **3** is oxoglaucine (O-methylatheroline). This compound has been isolated previously from the genus *Glaucium* [33].

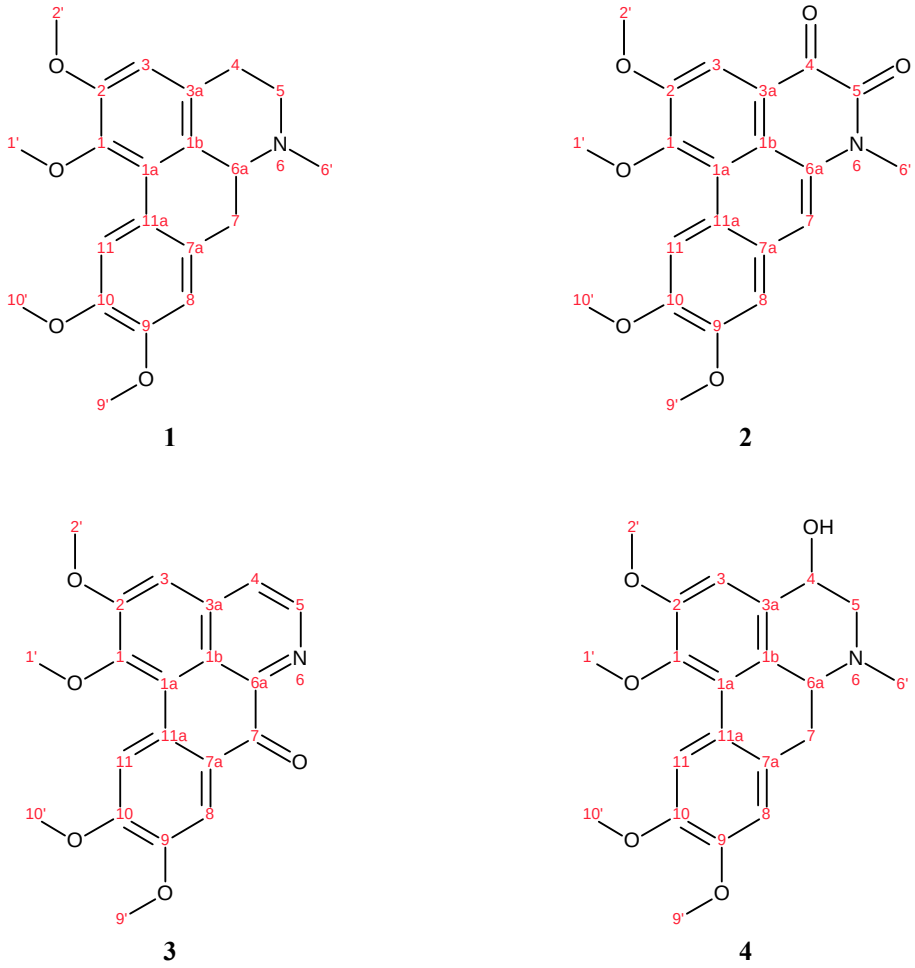

Figure 2: The isolated four isoquinoline alkaloids from *G. falvum*. **1**: Glaucine; **2**: Pontevedrine; **3**: Oxoglaucine or O-methylatheroline;**4**: Catalane.

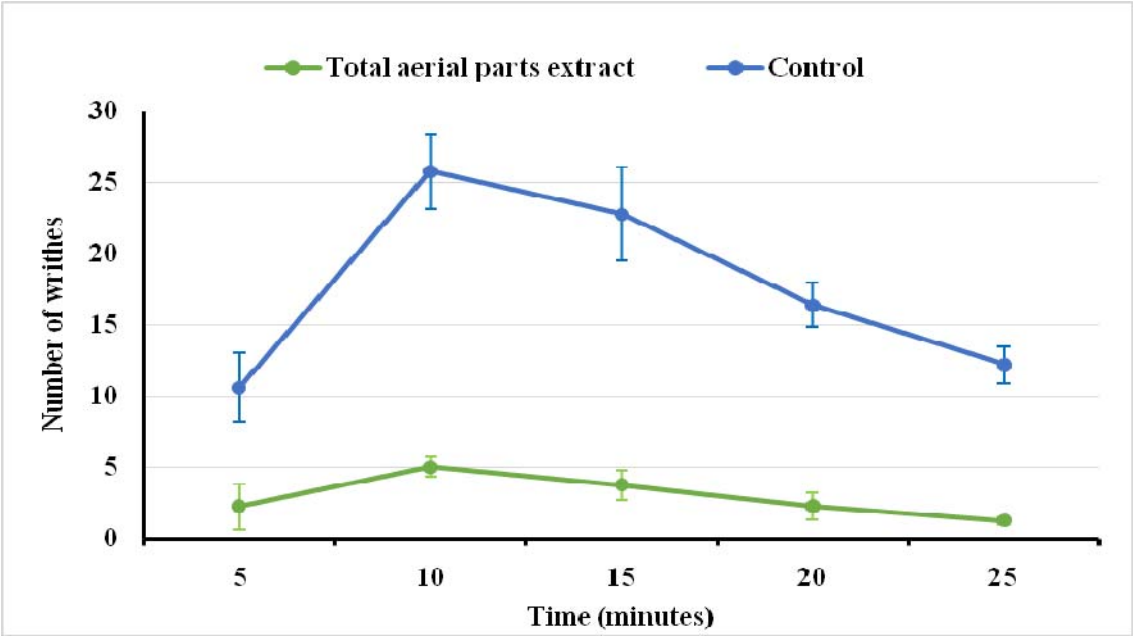

Figure 3: Evaluation of the analgesic activity of the ethanolic extract of the aerial parts of *G. flavum* Cr. (40 mg/kg, orally) on an acetic acid-induced writhing model in mice.

The EI-MS spectrum of compound **4**showed a molecular ion peak at m/z 370 (57.5%), which is compatible with the molecular formula, $C_{21}H_{25}NO_5$. The ^{1}H-NMR spectrum of compound **4** (Table 1) showed the presence of a triplet at δ 4.30 ppm (1H) and a doublet at δ 4.60 ppm (2H), indicating two methylenes at positions 4 and 5; as such, one of their protons was replaced by an electron-withdrawing group such as hydroxyl. Another triplet at δ 2.9 ppm and the doublet of a doublet at δ 3.00 ppm indicated that there was no unsaturation at positions 6a and 7. Four proton singlets at δ 3.64, 3.76, 3.81, and 3.84 ppm were assigned to four methoxy groups, and the aromatic protons appeared as singlets at δ 6.92, 6.97, and 7.86 ppm. The ^{13}C-NMR spectrum of compound **4**(Table 1) suggested the presence of 21 carbons. No carbonyl carbons were noticed in the spectrum. When comparing the data reported herein with those in the available literature,[25] proposed that compound **4** was cataline.

Table 1: ^{1}H-NMR and ^{13}C-NMR for the four isolated isoquinoline alkaloids. ^{1}H-NMR and ^{13}C-NMR was conducted in DMSO-d_6 at 600MHz and 150 MHz, respectively. The ^{1}H-NMR data were expressed as follows: δ_H (Number of protons, Multiplicity, *J*=Hz), For carbon numbering, refer to Figure 2.

Carbon number	Compound 1		Compound 2		Compound 3		Compound 4	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1	-	144.42	-	148.83	-	149.19	-	144.77
1a	-	123.07	-	123.68	-	118.31	-	122.82
1b	-	126.10	-	127.92	-	120.90	-	125.14
2	-	152.99	-	153.20	-	156.41	-	153.08
3	6.88 (1H, s)	111.01	7.61 (1H, s)	108.06	7.67 (1H, s)	107.29	6.92 (1H, s)	111.02
3a	-	126.25	-	120.29	-	135.08	-	125.76
4	2.98 (2H, dd, *J*= 17.4 & 3.0 Hz)	25.65	-	175.23	8.08 (1H, d, *J*=4.8 Hz)	123.90	4.30 (1H, t,*J*=5.4 Hz)	69.63
5	3.40 (2H, dd, *J*= 13.5 & 3.6 Hz)	51.45	-	155.62	8.84 (1H, d, *J*=4.8 Hz)	144.54	4.60 (2H, d, *J*=13.2 Hz)	53.00
6	-	-	-	-	-	-	-	-
6a	2.70 (1H, t, *J*=13.8 Hz)	61.15	-	117.93	-	144.67	2.90 (1H, t, *J*=13.8 Hz)	71.27
7	3.13-3.19 (2H, m)	30.31	7.86 (1H, s)	112.00	-	180.09	3.00 (2H, dd, *J*=18.6 &4.8 Hz)	67.73
7a	-	126.31	-	123.45	-	126.13	-	126.56
8	6.99 (1H, s)	111.53	8.14 (1H, s)	109.48	8.75 (1H, s)	109.04	6.97 (1H, s)	111.50
9	-	148.41	-	152.12	-	151.04	-	148.47
10	-	147.60	-	149.50	-	153.54	-	147.69
11	7.87 (1H, s)	111.73	8.99 (1H, s)	114.10	7.82 (1H, s)	110.06	7.86 (1H, s)	111.54
11a	-	120.80	-	130.32	-	128.57	-	119.55
1′	3.62 (3H, s)	55.43	3.94 (3H, s)	55.35	3.94 (3H, s)	60.39	3.64 (3H, s)	55.84
2′	3.80 (3H, s)	55.83	4.07 (3H, s)	56.47	4.02 (3H, s)	55.65	3.81 (3H, s)	60.14
6′	3.07 (3H, s)	40.94	3.73 (3H, s)	30.20	-	-	2.95 (3H, s)	55.57
9′	3.83 (3H, s)	59.72	4.08 (3H, s)	60.23	4.06 (3H, s)	56.42	3.84 (3H, s)	63.83
10′	3.77 (3H, s)	55.54	3.97 (3H, s)	55.47	3.99 (3H, s)	56.53	3.76 (3H, s)	59.83

ppm: parts per million, **s:** singlet, **d:** doublet, **dd:** doublet of doublet, **t:** triplet, **m:** multiplet, **J:** coupling constant

Pharmacological activities of G. flavum aerial parts

The ethanol extract was tested for its analgesic activity using the acetic acid-induced writhing technique [14]. The results, as shown in Figure 3, indicated that the ethanol extract at a dose of 40 mg/kg, exhibited an 84.62% reduction in writhing when compared to the control. Glaucine reportedly has analgesic [34] and antipyretic [26] activities, which could explain the analgesic activity exhibited by the extract.

The pretreatment of rats with a dose of 40 mg/kg of the extract significantly decreased the rats' hind paw edema thickness when compared to the control group (Figure 4A). The effects of the plant extract showed a rapid onset with a long duration of action in a pattern comparable to the anti-inflammatory activity of diclofenac sodium. In addition, the results obtained from the AUC calculation (Figure 4B) showed that the potency of the anti-inflammatory activity of the extract (40 mg/kg) is biologically equivalent to that of diclofenac sodium (4 mg/kg). The anti-inflammatory activity of the extracts could be, at least partly, ascribed to the presence of glaucine [28, 29, 34].

A cell viability assay was performed to evaluate the cytotoxic activity of the extract. The results, (Figure 5), indicated weak to moderate cytotoxicity against MCF-7, HeLa, and HEp-2 cells, and good activity against HepG2 and HCT cells with an IC_{50} of 28.3 and 33.2 µg, respectively.

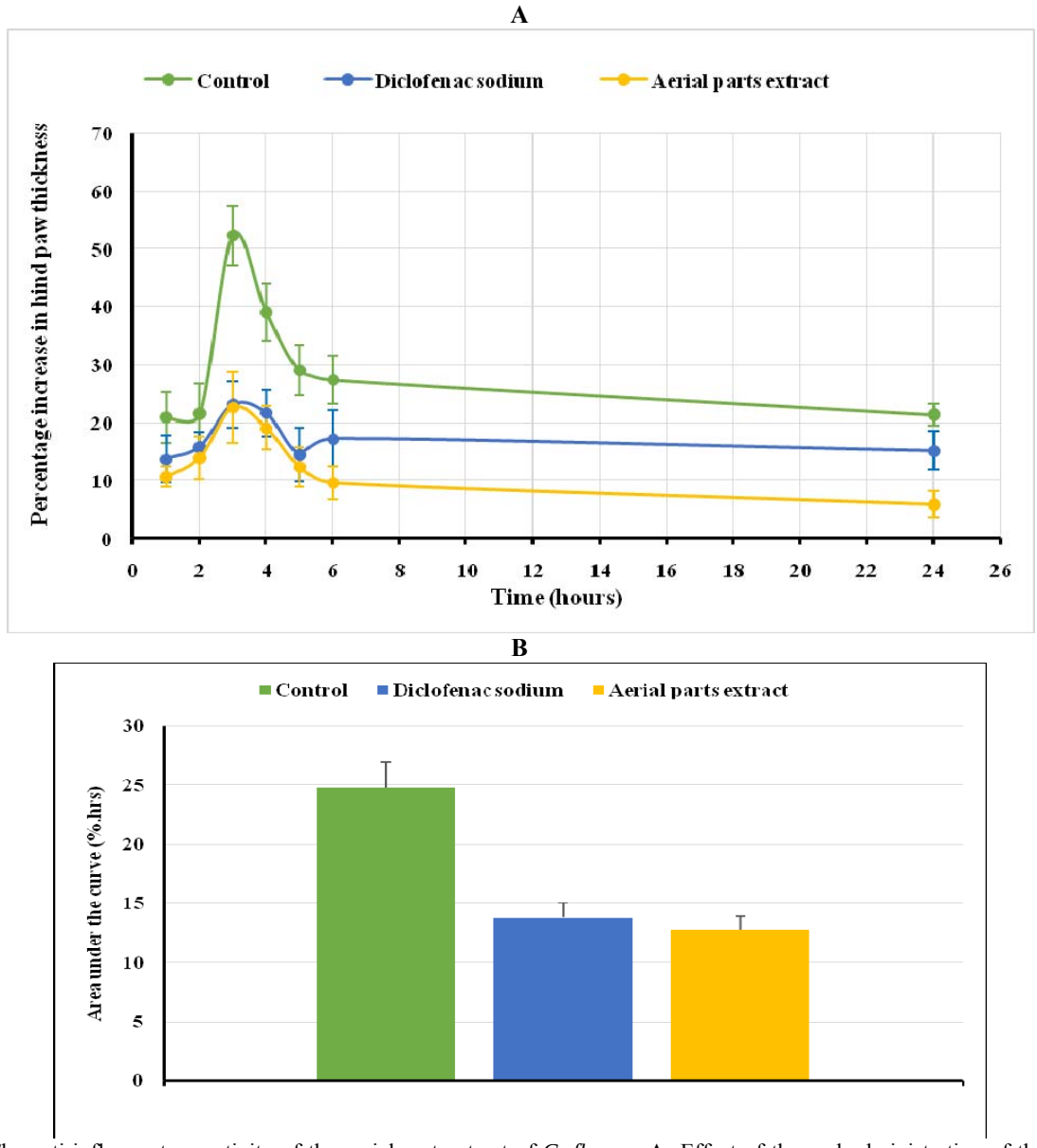

Figure 4: The anti-inflammatory activity of the aerial part extract of *G. flavum*. **A:** Effect of the oral administration of the *G. flavum* **aerial part extract** (40 mg/kg), **diclofenac sodium** (4 mg/kg, as a standard), on the percentage increase in hind paw thickness at different time intervals following the induction of edema using carrageenan; this effect was compared with the untreated edema observed in **controls**. **B:** The area under the curve (in A) was calculated for the **aerial part extract** (40 mg/kg), **diclofenac sodium** (4 mg/kg, as a standard), and **controls** in untreated rats. The area under the curve was calculated using the trapezoidal method.

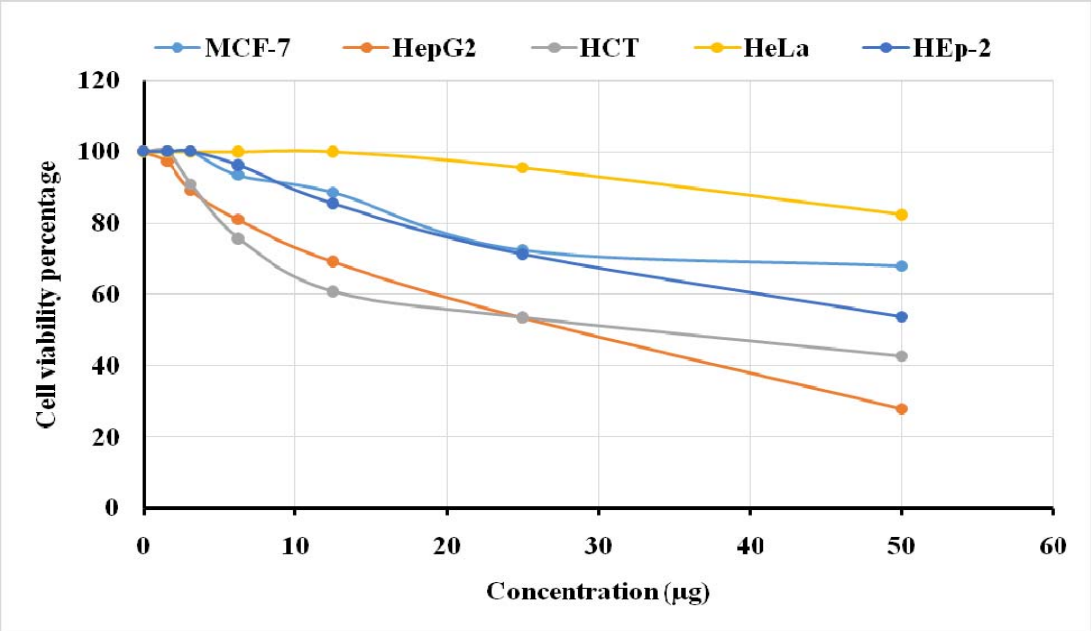

Figure 5: Cytotoxic activity of the *G. flavum* aerial part extract on MCF-7, HepG2, HCT, HeLa, and HEp-2 cell lines.

The antibacterial and antifungal activities of the extract was determined, and the results (Table 2) indicted that the extract had a significant antifungal and antibacterial effect against the tested microorganisms, except for *Aspergillus fumigatus* (RCMB 02568) and *Pseudomonas aeruginosa* (RCMB 010043), which is in comparison to amphotericin B, ampicillin, andgentamicin as standards. The antimicrobial activity of the extract could be attributed to the activity of individual components such as glaucine [35, 36] and oxoglaucine [26].

The variety of *G. flavum* growing widely in Egypt did not vary to a great degree, neither in terms of its constituents, nor with respect to its medicinal activities from other varieties growing in Europe and other parts of the world [5, 37, 38]. This conclusion indicates that the Egyptian wild yellow horn poppy is a valuable medicinal herb and an excellent source of the valuable alkaloid, glaucine. This herb should be exploited further in pharmaceutical preparations and, at the same time, it should be regarded as an endangered species by the Egyptian authorities and natural environment-preserving organizations.

Future work should involve genotyping the Egyptian variety of the yellow horned poppy using techniques such as restriction fragment length polymorphism identification (RFLPI) or random amplified polymorphic detection (RAPD) to relate the plant to other varieties in Europe and all over the world. A complete metabolite profile should be prepared using HPLC-MS techniques, especially for alkaloids, which will provide deep insights into the metabolite background of the plant and will help in its pharmaceutical processing and standardization.

Table 2: Antibacterial and antifungal activities *G. flavum*ethanolic aerial parts extract. The results are calculated as the inhibition zone diameter formed around the well (mm). The results are expressed as the mean ± SE of the mean for three independent experiments. NA: No activity was detected.

Microorganism name (strain number)	Inhibition zone diameter formed around the well (mm)			
	Aerial parts ethanolic extract		Standard	
	Mean	SE	Mean	SE
Fungi			**Amphotericin B**	
Aspergillus fumigatus (RCMB 02568)	NA	NA	23.7	0.1
Geotricum candidum (RCMB 05097)	13.8	0.37	28.7	0.22
Gram positive bacteria			**Ampicillin**	
Staphylococcus aureus (RCMB 010028)	16.2	0.58	27.4	0.18
Bacillus subtilis (RCMB 010067)	18.5	0.25	32.4	0.1
Gram negative bacteria			**Gentamicin**	
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	17.3	0.15
Escherichia coli (RCMB 010052)	15.2	0.63	22.3	0.18
Salmonella typhimurium (RCMB 010072)	14.6	0.44	25.4	0.18
Klebsiella pneumoniae (RCMB 000111)	15.6	0.37	20.2	0.25

CONCLUSION

This study described the isolation and structural elucidation of four isoquinoline alkaloids – glaucine, pontevedrine, oxoglaucine, and catalane – from the acidified methylene chloride fraction of the ethanol extract of the aerial part of the Egyptian yellow horn poppy (*G. flavum*). Some of the biological, medicinal, and pharmacological activities exhibited by the extract have been include anti-inflammatory, analgesic, cytotoxic, and antimicrobial activities. This study shed light on a plant that is classified as rare and endangered in the Egyptian flora due to urban sprawl, especially along the northern coast. This plant possesses many medicinally valuable secondary metabolites and retains many medicinal activities, which makes it worthy of addition on the red list of threatened species published by the International Union for Conservation of Nature (IUCN) (http://www.iucnredlist.org/). This plant should receive greater attention by the Egyptian authorities and civil community organizations working to preserve the environment in Egypt and around the world, particularly due to the plant's high value and unique secondary metabolite production machinery.

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